DEVICE FOR MAGNETIC IMMOBILIZATION OF CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/334,593, filed Dec. 3, 2001, and U.S. Provisional Application No. 60/307,843, filed Jul. 27, 2001.

FIELD OF THE INVENTION

The present invention relates to methods and devices that are useful in immobilizing, arraying and/or isolating cells individually. In particular, the present invention relates to methods and devices that are useful for immobilizing, arraying and/or isolating cells using a magnetic source.

BACKGROUND OF THE INVENTION

Many biological techniques employed in biotechnology, microbiology, clinical diagnostics and treatment, in vitro fertilization, hematology and pathology, require such processes as identification, separation, culturing, immobilization and/or manipulation of a target entity. Typical target entities include cells or microbes within a fluid medium such as culture fluids, environmental samples, blood or other bodily fluids. It is often desirable to retain viability of the target entity or to culture the target entity. When screening individual cells in a heterogenous population, it is desirable to array cells at discrete and separable locations. For example, an array of genotypically or phenotypically diverse cells would allow the investigator to rapidly perform large numbers of automated assays and observe results at the single cell level. The cell of interest could then also be further isolated and clonally expanded.

Isolation techniques typically involve labeling the target entity with a reagent that can be selected according to a characteristic property. Entities such as eukaryotic cells or certain microbes may be sorted using fluorescence-labeled monoclonal antibodies (Mabs) that are specific to a particular class of cells or microbes. Fluorescence Activated Cells Sorting (FACS) allows cells to be separated into different pools based on their reactivity to specific fluorescent Mabs. However, sorting cells into pools does not allow the investigator to experiment on or manipulate individual cells.

Manipulation of individual target cells required by certain biological techniques may involve such processes as insertion of genetic material, subcellular components, viruses, or other foreign materials or bodies into the target entities. In techniques such as transfection, cell injection or in vitro fertilization, mechanical probes or arms are often used to hold target cells in place. Such mechanical holding methods tend to obscure or damage the target cells. Experiments on isolated and immobilized individual cells also include hybridoma screening, patch-clamp experiments, single cell PCR and the like.

Devices and methods for precise non-destructive immobilization of specific individual target entities in an array of discrete locations, especially in an inexpensive and rapid manner are desirable.

Magnetic-based systems are commonly used to isolate and immobilize cells and rely on the use of cell-binding magnetic beads and a mechanism for cell capture. Although current magnetic-based cell separation methods afford certain advantages in performing medical or biological analyses based on biospecific affinity reactions involving colloidal magnetic particles, the systems developed to date are not particularly suited for immobilization or micromanipulation of individual cells.

A currently used method to isolate and immobilize cells involves placing steel wool inside a collecting vessel and then placing the vessel inside a strong magnetic field. A cell-containing fluid is mixed with magnetic beads that can specifically or non-specifically bind cells. The magnetic beads are generally coated with an antibody or compound that non-specifically binds cells. An operator pours the cell-containing fluid mixture through a magnetically activated cell sorting (MACS) magnetic filter that collects cells bound to magnetic particles. For example, a MACS device made by Miltenyi Biotec GmbH, Gladbach, Germany, employs a column filled with a non-rigid steel wool matrix in cooperation with a permanent magnet. In operation, the enhanced magnetic field gradient produced in the vicinity of the steel wool matrix attracts and retains the magnetic particles, while the non-magnetic components of the test medium pass through the column. It has been found that the steel wool matrix of such prior art high-gradient magnetic separation (HGMS) devices often causes non-specific entrapment of biological entities other than target entities. The entrapped non-magnetic components cannot be removed completely without extensive washing and resuspension of the particles bearing the target substance. Moreover, sizes of the columns in many of the prior art HGMS devices require substantial volumes of test media, which poses an impediment to their use in performing various laboratory-scale separations. In addition, the steel wool matrix may damage sensitive cell types. Furthermore, immobilizing target cells on steel wool does not allow the investigator to experiment on or manipulate individual target cells.

Another method for magnetically sorting and isolating cells has been to place bent metal pins inside microtiter wells and then move the holder for the microtiter wells inside a strong magnetic field. In the presence of the enhanced magnetic gradients, cells decorated with magnetic beads can be captured from any fluid samples inside the vessel or microtiter wells onto the bent metal pins. After the magnetic fields are removed, the captured magnetic beads can be removed from the bent pins by various techniques. This technique is primarily a batch process. As above, immobilizing target cells on bent metal pins does not allow the investigator to immobilize, array and/or isolate individual target cells.

Magnetic-based isolators are also used to purify nucleic acid or proteins from mixed samples, however, none can immobilize, array and/or isolate cells for study. Those magnetic-based cell isolators are intended to purify certain types of cells or molecules away from a background of other cells or molecules, respectively.

Thus, it is seen that there is a need for a magnetic-based system that can rapidly and efficiently immobilize, array and/or isolate target cells associated with magnetic particles from fluid samples on a substrate.

SUMMARY OF THE INVENTION

The invention provides for a device for immobilizing cells associated with magnetic material in a cell-containing fluid comprising a substrate having one or more magnetic receptacle(s), and a cell delivery device; wherein the magnetic receptacle comprises a permanent magnet and a localized magnetic field gradient.